Balancing Diets for Intestinal Protein Digestibility in Lactating Dairy Cattle

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Courtney K. Hoff
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Michael Van Amburgh

Abstract

The excessive use of nitrogen (N) has a negative impact on the environment and increases feed costs for dairy farmers. Current evidence suggests the metabolizable protein (MP) requirements for lactating dairy cattle can be met at much lower dietary crude protein levels than are currently in use in the field. However, the intestinal digestibility (ID) of feed N should be known to formulate closer to these refined requirements. An assay (Ross et al. 2013) that predicts the ID of feeds using an approach that more closely mimics ruminant digestion than other methods has been developed. The objective of this study was to determine if the ID assay of Ross et al. (2013) incorporated into a formulation model will allow us to accurately formulate for intestinal N digestibility in lactating dairy cattle using different animal protein sources. Multiparous and primiparous cattle (n=96) between 80-150 days in milk were balanced between the two treatments in a replicated pen design of 16 cattle per pen, 3 pens per treatment in a trial that was conducted over 63 days. Milk yield and dry matter intakes were measured daily, while milk yield and milk components, body weight, and body condition score were measured weekly. Two treatments were formulated for high and lower ID, using a blend of blood meal (BM) and with feather meal (FM) that was analyzed with the Ross assay. The BM was 74.6% N ID and the FM was 54.6% N ID. The high ID diet was formulated with 1.18 kg BM, and the lower ID diet was formulated with 1.3 kg of a blend of 82.8% FM and 17.2% of the BM. The BM was 74.6% N ID whereas the FM was 54.6% N ID, thus the high ID diet was formulated with 1.18 kg BM, and the low ID diet was formulated with 1.3 kg of a blend of 82.8% FM and 17.2% of the BM. The metabolizable energy allowable milk was 46.9 kg/d for both diets. Using data from the Ross assay, the MP allowable milk was 46.0 kg/d and 42.5 kg/d, for high ID and low ID treatments, respectively. For comparison, using ID from a cecetomized rooster assay (Boucher et al. 2009), predicted MP allowable milk was 46.0 kg/d and

50.3 kg/d for high ID and low ID treatments, respectively. Data were analyzed using JMP and the Mixed Model procedure. Observed milk yield was 44.5 kg/d for high ID diet and 43.1 kg/d for low ID diet and energy corrected milk yield was 49.5 and 46.2 kg ($P = 0.04$). These results indicate that the Ross ID assay can be useful in diet formulation to improve predictions from nutrition models and that feather meal is less digestible in cattle than in a chicken model.

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List of Abbreviations

AA	Amino Acids
ADIN	Acid Detergent Insoluble Nitrogen
AF	As Fed
BCS	Body Condition Score
BM	Blood Meal
BW	Body Weight
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude Protein
DIM	Days in Milk
DM	Dry Matter
DMI	Dry Matter Intake
ECM	Energy Corrected Milk
FM	Feather Meal
GIT	Gastrointestinal Tract
HID	High Intestinal Digestibility (treatment)
ID	Intestinal Digestibility
IVNIDA	*In Vitro* Nitrogen Intestinal Digestibility Assay
LID	Low Intestinal Digestibility (treatment)
ME	Metabolizable Energy
MP	Metabolizable Protein
N	Nitrogen
NPN	Non-Protein Nitrogen
NRC	National Research Council
PUN	Plasma Urea Nitrogen
RDP	Rumen Degradable Protein
RUP	Rumen Undegradable Protein
TMR	Total Mixed Ration
uN	Unavailable Nitrogen

Introduction

Feeding protein to lactating dairy cattle is expensive but necessary for productivity, and might increase the excretion of nitrogen (N) into the environment if protein sources are overfed or of low quality or digestibility. Nutritionally and biochemically, dietary protein is defined as a sequence of amino acids. Currently, protein is evaluated by the amount of N in feed and expressed as crude protein (CP), however this measure fails to capture the intrinsic characteristics of the feed such as digestibility, which is essential to meet the dietary needs of cattle. Alternatively, expressing protein supply in terms of metabolizable protein (MP) would help to formulate dairy diets with much higher accuracy without having to overfeed total CP, as it accounts for the digestible true protein that is available to the animal. As the MP requirement for high producing dairy cattle continues to be refined, we will need to accurately characterize the total digestibility of protein of common feedstuffs provided in dairy cattle diets. Historically, the indigestible fraction of protein was described as acid detergent insoluble nitrogen (ADIN), (Pichard and Van Soest, 1977; Sniffen et al., 1992). The ADIN is the fraction of protein bound to the acid detergent fiber in plant and other cellulosic-based materials. However, ADIN is difficult to measure accurately because of the methodology, due to the fact that it relies on N being bound to plant fiber. Therefore, it cannot be effectively used to determine undigested N in feedstuffs lacking fiber, such as animal based protein supplements. In 2013, Ross and coworkers proposed an assay that produced a more reliable measure of the indigestible N fraction of feeds relative to ADIN. This assay, dubbed the *in vitro* nitrogen intestinal digestibility assay (IVNIDA), has been shown to accurately predict the behavior of cattle fed lower protein diets that were formulated with ID as a limiting factor (Higgs et al. 2013; Gutierrez-Botero 2015). However, there are byproduct feeds like feather meal (FM) used in cattle diets that have been analyzed using alternative methods such as the pepsin-HCl assay

(AOAC, 1999) or the cecectomized rooster assay (Titgemeyer et al., 1990), however these assays may not be appropriate for ruminants. The pepsin-HCl assay does not adequately mimic the digestive system found in ruminants because the pepsin concentration used is too high resulting in over-estimated digestibility. The digestion in the cecetomized rooster is also impractical as the rooster has enzymes like elastase that are not present in cattle and will also result in overestimated digestibilities when applied to cattle. Previous work to determine digestibility of FM suggested that it was poorly digested when analyzed using the Ross et al. (2013) assay and results showed high digestibility using the cecectomized rooster assay (Boucher et al. 2009) and the pepsin-HCl method (AOAC, 1999). This has led to significant controversy because there are millions of kilograms of FM processed and fed to cattle in the U.S. every year, so both economic and environmental decisions are being questioned by the dichotomy of assay outcomes.

Our primary objective was to determine if the ID assay of Ross et al. (2013) as incorporated into the CNCPS v6.5 will allow us to accurately formulate for intestinal N digestibility in lactating dairy cattle under isocaloric and isonitrogenous dietary conditions. Feather meal has low digestibility due to the keratinize protein that makes up the feathers, thus predicted digestibility is conditional on the assay enzymes used and pH of the buffer media, thus this feed is difficult to characterize for all animals since digestive systems vary. When evaluated with the Ross assay, FM has a low digestibility, but when evaluated with the cecectomized rooster assay, FM was reported to have a high ID (Boucher et al. 2009). Because values obtained with these two assays are not in agreement, this allows us to test the accuracy of the proposed in vitro assay against another commonly accepted model within the actual lactating cow. Thus, our secondary objective then became to evaluate the performance of lactating dairy cattle on different animal protein sources, BM and FM. In this study, we formulated diets with CNCPS based on the IDs obtained

from the Ross assay to compare the performance of BM, a known high ID protein source, versus FM, which has a disputed ID. By formulating the diets in CNCPS we can predict the milk production based on the MP provided to the cattle. While accounting for the differences in digestibility for the different assays and then compare predictions to actual cattle performance.

We expect that the cows will behave based on the predictions of the Ross assay data for protein ID, and that cows fed the high intestinal digestibility (HID) protein mix will outperform cows fed the low intestinal digestibility (LID) protein mix, which includes FM. We hypothesize that there will be a significant difference between treatments for performance parameters such as milk yield, energy corrected milk (ECM), and fat and protein milk components due to the difference in ID between the diets. Because the diets are formed under isocaloric conditions, we predict no significant differences between dry matter intake (DMI), body weight (BW), and body condition score.

Review of Literature

Defining N in Feedstuffs

As the public continues to scrutinize the dairy industry and the associated environmental impact of milk production, it becomes increasingly important for dairy farmers to evaluate and control nutrient inputs and outputs. Nitrogen is a nutrient that could benefit from redefining, as the current approach for diet formulation often causes it to be fed in excess of requirement and is consequently lost to the environment. The feeding of excess N has a negative impact on the environment and increases feed costs for dairy businesses.

The protein content of feed is currently described as CP, and calculated by multiplying total N content by the constant factor 6.25. This definition is not descriptive of individual feeds and does not directly address any of the needs of the cow, because it includes all N, regardless of whether or not it is actually digestible by the animal. The cow has two distinct N requirements – ammonia to support protein synthesis in rumen microbes, and amino acids (AA) that are generated by the intestinal digestion of protein that bypassed digestion in the rumen, otherwise known as rumen escape proteins. This fraction that is digested by the cow in the intestine is collectively known as metabolizable protein (MP). The MP is generally absorbed in the small intestine, and includes not only the AA in the feed protein that escapes the rumen, but also microbial protein from the rumen and endogenous protein sloughed from the gastrointestinal tract (GIT). Milk protein synthesis is a function of energy supply and AA balance and CP does not adequately capture either of these aspects. Due to this disparity, while cattle might be receiving high CP diets they might not be receiving adequate MP or AA to match the metabolizable energy (ME) allowable milk available in the diet. Alternatively, ME allowable milk, which is the amount of energy in the diet to allow the cattle to synthesize milk based on predictions of the CNCPS, might not be

met before MP allowable milk making the diet energy limited. Diets are typically formulated with ample amounts of energy for high production lactating dairy cattle, thus the diets can be N or AA limiting despite high CP intake. If fed a diet balanced for AA and ammonia and digestibility, instead of using a more static value such as CP, MP allowable milk could be more accurately predicted, and the total CP of the diets could be decreased, leading to a decline excess N excretion.

As total N intake decreases, the ID of feed protein sources within the diet becomes a potentially limiting factor. The Cornell Net Carbohydrate and Protein System (Fox et al., 2004; Van Amburgh et al., 2015) has used an approach to estimate the intestinal availability of feed proteins since the development of the CNCPS model. There are two fractions of digestible protein described, rumen degradable protein (RDP) and rumen undegradable protein (RUP). Within the RUP, there is a fraction described as completely indigestible by the intestines. On most feed analyses, unavailable protein is often described by acid detergent insoluble nitrogen (ADIN) which is the portion of N that is bound to insoluble fiber, the acid detergent insoluble fiber. This was described by Goering et al. (1970) because ADIN was highly correlated with the apparent ID of forages. Although ADIN is used to describe the indigestible fraction of N in the National Research Council (NRC) recommendations for nutrient requirements in dairy cattle (NRC, 2001), the fraction was assigned a 5% ID factor because there was evidence that some N could be liberated and digested from this chemical fraction.

N Utilization in Dairy Cattle

Currently, on dairy farms the average efficiency of use of feed N in lactating dairy cattle range from 20-32 %, and the theoretical efficiency limit has been projected at 40-45% in lactating dairy cattle (Van Vuuren and Mejis, 1987; Hvelpund and Madsen 1995; Dijkstra, 2013). A practical

goal for dairies under the current N feeding system is 38-40% efficiency. As we move towards increased efficiency in N feeding, it is important to emphasize the quality and digestibility of feeds. If the feed inputs for N are redefined, farms will not resort to overfeeding CP to maintain high production and a greater efficiency of N use for high producing dairy cattle could be achieved.

To understand how much N a dairy cow requires one must understand how N is utilized in the cow and how N excesses or deficiencies are metabolically managed. Once it enters the cow there are several pathways protein N can take depending on whether it is solubilized in the rumen or escapes fermentation to be digested intact in the small intestine. When solubilized in the rumen N is utilized by rumen microbes to meet their requirements for growth and fermentation. In turn, soluble N is ultimately being incorporated into microbial protein. Post-ruminally, microbial protein and digested feed protein provides absorbable N for incorporation in milk and tissue production: muscle, organs, hair, and enzymes. If utilized as fuel by cells lining the GIT, it could be reused in the cow as endogenous N (Ouellet et al. 2010) when these cells internally slough, similar to microbial protein. Most of these forms have the ability to be internally recycled within the cow. There are four net outcomes for N utilization and they are milk, urine, feces and scurf, which is hair, skin and related tissues. Milk is the desirable output, with N being synthesized into whey, casein, and non-protein nitrogen (NPN) production that contribute to milk protein and components. It can also be excreted in feces as N originating from endogenous, microbial, and feed sources.

The N excreted in urine is generally in the form of urea as a waste product. Unlike fecal N, excretion of urinary N is variable, and responds to changes in N intake (Kauffman and St. Pierre, 2001; Van Amburgh et al., 2015). This is because ruminants have the unique capability to recycle N within their system as urea. Urea can be reabsorbed into the rumen and converted into ammonia and then used by rumen microbes as a source of N. Under conditions when the cow is being overfed

N, the cow does not necessarily need to recycle N back through the GIT because there is adequate feed protein available at all times. Accordingly, excess N is converted to urea to be excreted instead via the kidney. However, if N is not in excess the cow will recycle urea N and will make the decision to return the urea to circulation for distribution to the GIT for microbial utilization (Reynolds and Kristensen, 2008). By feeding appropriate amounts of N, it can encourage the cow to recycle N which will enhance the efficiency of use of intake N and decrease excretion by being captured by rumen microbes as it recycles. Thus, urinary urea N is a good indicator of the N status of a cow because the amount of urea excreted in the urine is a function of the amount and form of N fed to the cow. Any N that is not required for urea recycling is excreted in the urine (Van Amburgh et al., 2015).

Productive N is the portion of the N that is utilized for desirable outcomes in the cow, such as milk production, growth, and gestation. The ratio of productive N to urinary N can be a good indicator of how efficiently the cow is utilizing fed N, and ideally the ratio should be equal to 1:1 in efficient herds (Van Amburgh, 2017). For example, the CNCPS can be used to accurately predict urinary N excretion (Higgs et al. 2012), thus if the urinary N excretion is greater than the milk protein N excretion, the cow is wasting N. To evaluate this situation, if productive N is lower than urinary N, it means the CP being fed is either exceeding the cattle requirements or is not in a source readily available to the cow. This efficiency could be improved by feeding a more digestible protein or by decreasing the total amount of CP in the diet. Another measure that could potentially be used to evaluate N use is the relationship between intake N and milk N.

Indigestible N

Consistent with all other formulating systems, the CNCPS has assigned the ADIN as the unavailable feed protein and assigned a 0% ID to this fraction. Based on the 5% digestibility

given to ADIN by the NRC committee (NRC, 2001), there was an opportunity to more accurately describe this component of protein supply since it greatly impacts the ability to refine N feeding and improve the prediction of AA availability. More contemporary work has suggested this static approach does not account for the variation in digestibility, which results in inconsistent cattle performance relative to the predicted values due to deficiencies in MP supply.

Several approaches have been developed to predict the ID of protein in feeds and are a departure from the ADIN determination system of feed chemical composition (Calsamiglia and Stern, 1995; Ross et al., 2013). The three-step method described by Calsamiglia and Stern (1995) was one of the first methods that tried to describe the ID of proteins. Other assays for N indigestibility include the pepsin-HCl assay (AOAC, 1999) and the cecectomized rooster assay (Parsons, 1985; Titgemeyer et al., 1990; Boucher et al. 2009) which have been used successfully for a variety of proteinaceous feeds mostly for poultry and swine. The assays have also been used to evaluate digestibility for ruminants and comparisons have suggested that the cecectomized rooster is a viable alternative to predict ID of proteins in cattle. However, the cecetomized rooster assay has not been prospectively evaluated using lactating dairy cattle fed N limited diets and the predictions were only accomplished through comparisons of in vitro testing versus the rooster assay (Boucher et al. 2009). The cecectomized rooster assay is conducted by feeding cecectomized birds feeds that have undergone ruminal digestion. The feeds are first ground through a 1mm screen before undergoing ruminal fermentation in situ within a cannulated cow. The feeds are then intubated past the crop to undergo intestinal digestion. Excreta is collected, lypholized, and analyzed for N to determine ID of proteins (Boucher et al, 2009). However, the presence of a different enzyme mix in the cecectomized rooster may not convey accurate ID for use in ruminant nutrition. This is especially the case for animal proteins, such as FM. Feather meal is high in keratin, a protein that

has a similar composition as the protein elastin (Ferraro et al. 2016). Elastin is primarily broken down by the enzyme elastase and elastase is present in the chicken digestive tract but is not in same form in the cow. The presence of elastase might increase digestibility of keratin in the chicken tract, but because elastase is not present in the cow's digestive tract, the cecectomized rooster assay might not accurately portray the cow's ability to digest feedstuffs containing keratin. Carboxypeptidase B is a pro-elastase enzyme normally found in cattle, however addition of this enzyme to the Ross assay at multiple levels was unable to alter FM digestibility in the Ross assay (Ross, personal communication), suggesting that it is unlikely that cattle have appropriate enzymes for digesting such substrates.

Ross et al. (2013) a procedure to estimate ID in ruminants and has incorporated this approach into CNCPS to better meet the predicted protein requirements of dairy cattle (Ross et al. 2013, Gutierrez-Botero et al. 2015; Fessenden et al. 2017). The Ross assay, otherwise known as IVNIDA, was developed specifically to be accessible to commercial labs. It is done entirely *in vitro* and requires rumen fluid, which is gathered from cannulated cattle and used to simulate ruminal digestion. There are a couple of key differences between the Ross assay and the other assays used to determine uN (undigested nitrogen). First, a main difference is that there is no use of bags in the ruminal digestion step. Many of the other assays, place bags in situ within a cannulated cow for ruminal fermentation. While developing the Ross assay method, bags were found to extend the lag time of ruminal fermentation, and the loss of soluble components of feed was too high – making results to variable. Instead, ruminal fermentation is performed in Erlenmeyer flasks (Ross et al 2013) in vitro. Second, the enzyme mix used is more comparable to the enzymatic activity that actually occurs in ruminants. For example, porcine peptin was included in the enzyme mix at a rate 60% of that in a previous assay design, to better mimic the enzymatic

activity of pepsin in ruminants (Ross et al. 2013). Also, because pancreatin results in other assays tended to have a higher variability, this was replaced with a mixture of trypsin, chymotrypsin, amylase, and lipase. Finally, instead of using trichloroacetic acid precipitation to terminate the digestion, filtration through glass microfiber filters was utilized to capture all undigested feed particles, and also allowed for the calculation of the amino acid fraction of uN, which other assays could not capture (Ross et al. 2013). The assay also corrects for the microbial protein that is introduced by the rumen fluid. This is done by using neutral detergent residue from corn silage because washed corn silage has a very low N content and the silage also readily sustains bacterial growth, thus any increase in N content is associated only with bacteria (Ross et al. 2013). These aspects of the Ross assay allow for a more accurate prediction of N availability from digested feed in the cow. The Ross method has been evaluated and has demonstrated increased accuracy in lactating cow models than other assays of ID to predict MP allowable milk yield (Gutierrez-Botero et al. 2015; Fessenden et al. 2017). The results of cattle study described in Gutierrez-Botero et al. (2015) supported our desire to apply the ID and uN values in the CNCPS for diet formulation. The study of Gutierrez-Botero et al. (2015) was conducted by formulating two different diets for high producing cattle using two different BMs with different predicted ID to test the accuracy and precision of the assay (Ross et al., 2013). However, there are differences in assay predictions of ID based on animal model being used and these differences, especially in feather meal affect how the ingredient is used in diet formulation for ruminants, thus an actual evaluation in lactating dairy cattle was necessary to fully evaluate this dichotomy.

Conclusions

More accurate predictions of N availability in feeds should allow nutritionists to reduce the amount of N in diets while maintaining milk production, reducing feed cost and reducing the

amount of N that is not directly utilized by the cow. This will allow nutritionists to choose feeds that will deliver protein effectively. Currently, animal derived proteins and manufactured protein/AA mixes have been included in the diet to provide cattle access to high quality protein. While the inclusion rates of these feed additives are relatively low, the protein provided by them can have a large impact on the total N in the diet. There is potential to increase the amount of productive N the lactating dairy cow uses without excreting excess N as a waste into the environment.

Materials and Methods

Animals, Treatments, and Experimental Design

The Cornell University Animal Care and Use Committee approved all procedures involving the use of animals. Ninety-six cattle at the Cornell University Ruminant Center (Harford, NY), 84 multiparous (111.0 ± 32.2 days in milk (DIM), 55.2 ± 4.5 kg/d milk) and 12 primiparous (119.6 ± 32.9 DIM, 44.8 ± 2.6 kg/d milk) were blocked by DIM and milk production into 6 pens of 16 cattle (14 multiparous, 2 primiparous). Treatments were allocated to 3 consecutive pens for the convenience of the farm staff and milking routines through a two-sided 16-stall milking parlor. The trial took place between November 17, 2017 and January 16, 2018 and consisted of a 1-wk adaptation period, followed by a 1-wk covariate period. Cattle were housed in pens in a four row barn with one sand bed per cow and more than one headlock per cow and free access to water. Diets were maintained throughout this time period, but sample collection did not occur during the week of December 24, excluding daily milk yields which were provided by Alpro data system software in the milking parlor.

Treatment diets were formulated using CNCPS v6.5 based on the wet chemistry of the ingredients used in the diets (Table 1). The protein sources of interest in the two dietary treatments, BM and FM, were included into the diets at slightly different rates to maintain iso-nitrogenous diets (Table 2). In the HID diet the protein mix contained a protein source that was 100% high ID BM. In the LID treatment, the protein source in the mix was a blend of 82.8% FM and 17.2% the BM. The overall N content within the mixes was balanced because of the inclusion of BM in the LID mix – because FM alone would have had a lower total N. The ID of the mixes differed because the BM and FM were 25.38% and and 45.39% unavailable N respectively. The LID protein mix contained BM to make up for the difference in the total N content of FM. The composition of the

two diets for the treatments remained largely similar with the exception of the sources of protein in the protein mixes. The mixes were prepared in three batches at a local feed mill (Purina Animal Nutrition, Caledonia, NY), and delivered as needed to the farm. The full composition of the protein mixes is outlined in Table 2

Table 1. The formulated diet composition of the two diets, high intestinal digestibility (HID) and low intestinal digestibility (LID) with model inputs based on Ross assay.

	Treatment	
Ingredients, % dry matter (DM)	HID	LID
Corn silage WII	27.5	27.2
Corn silage TMF	27.5	27.1
Mixed mostly legume haylage	9.0	9.1
High moisture ear corn	9.8	9.7
HID Protein Mix	26.2	---
LID Protein Mix	---	26.3
Chemical Composition		
DM, % as fed (AF)	43.4	42.0
CP, %DM	15.1	14.7
NDF, %DM	30.2	28.5
ADF, %DM	18.4	17.2
Ca, %DM	0.79	0.77
P, %DM	0.46	0.44
ME*, mcal/kg DM	2.7	2.7
Methionine*, %MP	3.4	3.2
Lysine*, %MP	8.3	7.6
Lysine:Methionine*, %MP	2.4	2.4

*Calculated values from CNCPSv6.5

Table 2: The ingredient composition for the two diets expressed as a percent dry matter (%DM) of the diet.

	Treatment	
Ingredient, %DM	HID	LID
Corn silage WII	27.5	27.2
Corn silage TMF	27.5	27.2
Mixed mostly legume haylage	9.0	9.1
High moisture ear corn	9.8	9.7
Protein Mix Composition		
Wheat midds	6.1	6.1
Canola meal solvent	4.5	4.5
Energy Booster 100	2.5	2.5
Molasses dried	1.2	1.2
Sodium bicarbonate	0.9	0.9
Limestone, ground	1.1	1.1
Salt White	0.3	0.3
Dicalcium Phosphate	0.5	0.5
Urea 281 P	0.3	0.4
Smartamine M	0.1	0.1
Magnesium Ox	0.2	0.2
Sugar Sucrose	2.4	2.4
Soybean Meal 47.5 Solvent	1.6	1.6
Vitamin Premix ADE	0.2	0.2
Rumensin 80	<0.01	<0.01
AjiProL	---	0.2
Blood Meal Average	4.2	0.8
Feather Meal	---	3.9

Sampling Procedure

Cattle were milked three times per day at approximately 0800 h, 1600 h, and 0000 h with all data from each milking recorded by the Alpro herd management system (DeLaval International AB, SG). Milk samples for component testing were collected once per week for three consecutive milkings. The samples were preserved with 2-bromo-2-nitropane-1, 3-diol at 4°C until they were received by Dairy One (Ithaca, NY) for analysis.

Cattle were fed a total mixed ration (TMR) once per day, targeting refusals for 5-6%. The diets for each treatment were made in a single batch and distributed to the three pens assigned each treatment. The amount of feed that was offered and refused was recorded using Feed Watch

(Valley Agricultural Software, Tulare, CA). On farm the DM of feeds was determined once per week using a moisture tester (Koster Crop Tester Inc., Brunswick, OH) and the Feed Watch software was adjusted accordingly, intended to ascertain proper diet feeding. Weekly forage, TMR, and refusal samples were also collected and these were used for DM and feed analysis.

Once per week, cattle were weighed after their 1600 h milking using a platform scale XR3000 (Trutest, NZ). At the same, a body condition score (BCS) was assigned on a scale from 1-5 by using the average of assessments done by two evaluators.

Blood was drawn once per week via venipuncture of the coccygeal vein. Cattle were locked in headlocks upon returning from their 1600 h milking, disinfected with gauze soaked in alcohol solution and bled via vacuum tube. Tubes were centrifuged and plasma was extracted and frozen for potential use in plasma urea nitrogen (PUN) determination.

Sample Analysis

Milk samples were analyzed at Dairy One (Ithaca, NY) for fat, true protein, and total solids by mid-infrared methods Foss Milkoscan FT+, Foss Inc., Eden Praire, MN; AOAC, 1990). Forages, TMR, and refusals were dried in a forced air oven before being ground through a 2-mm screen using a Wiley Mill (Arthur H. Thomas, Philadelphia, PA). Samples were pooled as composites for each week of the trial and analyzed for DM at 105˚C (AOAC, 1990), NDF and ADF using heat stable α-amylase (Van Soest et al., 1991), total N, neutral detergent insoluble nitrogen and ADIN by combustion assay (Leco Instruments Inc; AOAC, 2000), fat (AOAC, 2006), starch (Hall, 2015) and sugar (Dubois et al., 1956) (Cumberland Valley Analytical Services, Hagerstown, MD).

The Ross (2013) IVNIDA was used to determine the uN of the BM and FM. These values were incorporated in the CNCPS to predict the ME and MP allowable milk. Milk yield was expressed

as both actual milk yield volume and ECM according to the Tyrell and Reid (1965) equation: ECM (kg) = (12.82 * kg fat) + (7.13 * kg true protein) + (0.0323 * kg milk).

Data were analyzed using the mixed effects model of JMP (v.13 SAS Institute, Inc., Cary, NC). The model included pen, animal, animal within pen, treatment, covariate period milk yield and parity. Least squares means were reported and significance was determined at a level of $P< 0.05$ while anything from $0.05<P< 0.10$ was considered a trend.

Results

Results of the Ross assay for ID of BM and FM were 74.6%N and 54.6%N, respectively (Table 3). The ID of FM determined by the cecectomized rooster assay of Boucher et al. (2009) is also reported, but ID of BM was not included because a similar BM was not analyzed in their experiment.

Dry matter intake for cattle on treatments HID and LID was 26.2 ± 0.4 kg/d and 25.7 ± 0.4 kg/d, respectively, and were not different (Table 4). Dry matter intake decreased during the duration of the study (Figure 1). There are missing data for Pen 301 (HID treatment) between weeks 5 and 7 due to a software error. Body weight did not change throughout the study for either treatment therefore statistics were only run on average weight, which did not differ. Body condition score did not change throughout the study for both treatment, and the scores remained at a BCS of 3.0 for the duration of the study.

Cattle fed the HID diet had a 1.4 kg/d higher milk yield, than cattle on the LID diet representing a trend (P=0.11) (Table 4). Milk yield decreased over weeks during the study (Figure 2). Cattle fed the HID diet had 3.3 kg/d higher ECM yield on average compared to the LID fed cattle (P=0.04), and this difference in ECM yield was maintained over the experiment (Figure 3). Milk fat percent and milk fat yield were both higher for cattle on the HID diet than cattle on the LID diet (P=0.04 and P=0.01, respectively), protein yield was higher (P<0.01), while protein percent tended to be higher for HID as well ($P=0.06$).

Model predictions from the CNCPS using the Ross assay inputs for ME allowable milk were greater than observed milk yields by 2.4 kg/d and 3.8 kg/d for HID and LID treatments, respectively (Table 5). Using the ID inputs derived from the cecectomized rooster assay, the model over-predicted MP allowable milk yield by 1.5 kg/d and 7.2 kg/d for the HID and LID diets

respectively. Using the Ross assay inputs in the model, MP allowable milk yield was over-predicted by 1.5 kg/d and under-predicted by 0.6 kg/d for HID and LID diets respectively. The estimated milk production using Ross assay inputs for the CNCPS model were closer to cattle performance compared to the cecectomized rooster assay inputs, especially for diets containing FM.

Table 3. The total N content and intestinal digestibility as determined by the Ross assay and the cecectomized rooster bioassay.

	Ross Assay		Cecectomizd Rooster	
	Total N, %DM	ID, %N*	Total N, %DM	ID, %N*
Blood Meal	16	74.6	16.5	-
Feather Meal	14.1	54.6	14.5	80

*ID, %N is the percentage of digestible N in feed

Table 4. The effect of high intestinal digestibility (HID) and low intestinal digestibility (LID) diets on dry matter intake (DMI), body weight (BW), milk, energy corrected milk (ECM) and milk components, fat and protein.

	HID	LID	s.e.	P
N	48	48	-	-
BW, kg	743	745	1.8	0.42
DMI, kg/d	26.2	25.7	0.4	0.48
Milk, kg/d	44.5	43.1	0.5	0.11
ECM, kg/d	49.5	46.2	1.7	0.04
Fat, %	4.08	3.81	0.10	0.04
Protein, %	3.03	2.93	0.04	0.06
Fat, kg/d	1.88	1.70	0.05	0.01
Protein, kg/d	1.39	1.31	0.01	<0.01

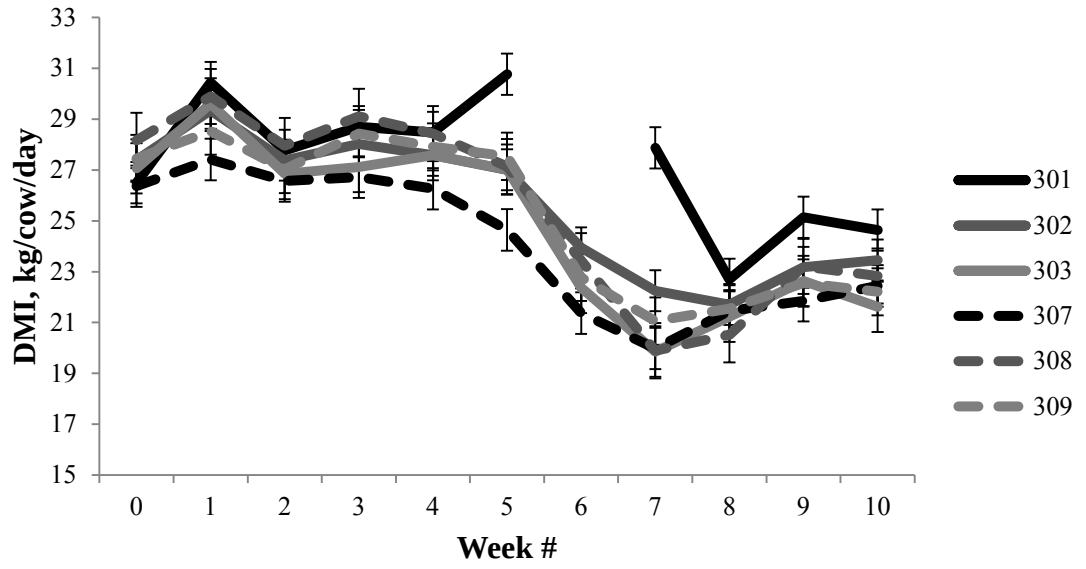

Figure 1. Average weekly dry matter intake (DMI) for high intestinal digestibility (HID) and low intestinal digestibility (LID) diets by week of experiment by pen. Solid lines represent the pens that were HID treatments, dashed lines LID.

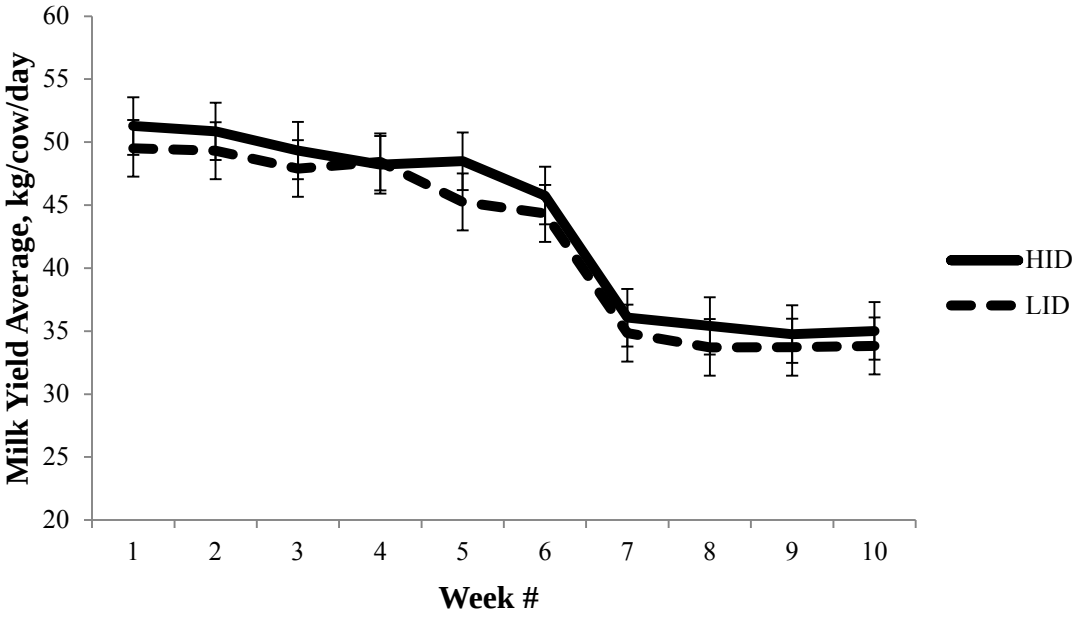

Figure 2. Average weekly milk yield for high intestinal digestibility (HID) and low intestinal digestibility (LID) diets by week of the experiment by treatment.

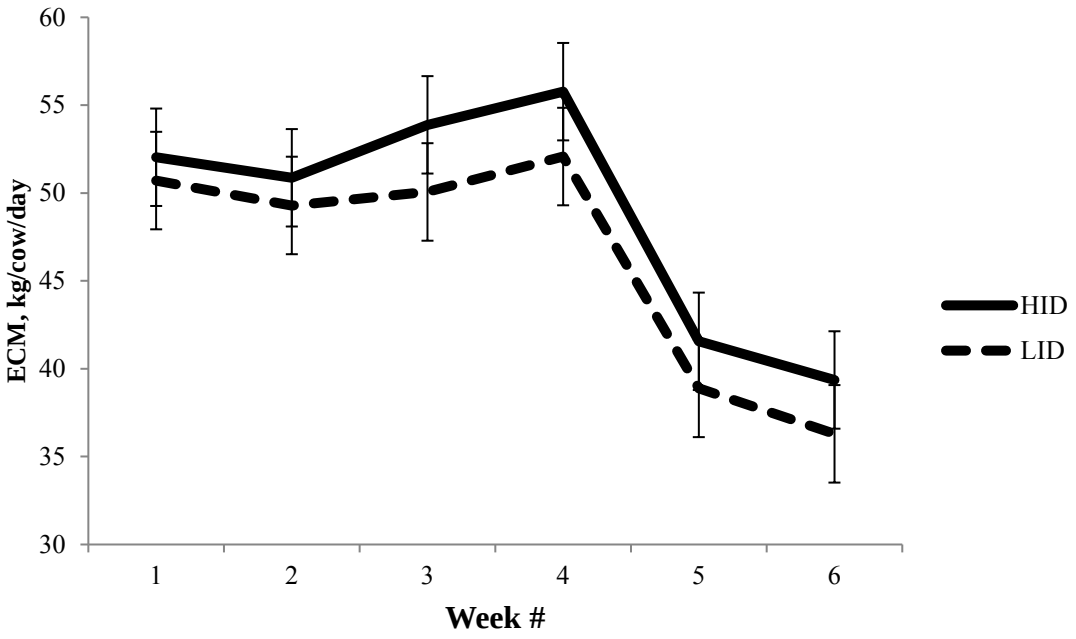

Figure 3. Average energy corrected milk (ECM) for high intestinal digestibility (HID) and low intestinal digestibility (LID) diets by week of the experiment and treatment.

Table 5. A comparison of the CNCPS model predicted MP and ME allowable milk based on assay inputs, the animal characteristics, the actual dry matter intake and complete feed chemistry for the high intestinal digestibility (HID) and low intestinal digestibility (LID) treatments.

	Treatment	
	HID	LID
Actual milk, kg	44.5	43.1
Predicted ME allowable milk, kg	46.9	46.9
Using Ross assay inputs		
Predicted MP allowable milk, kg	46.0	42.5
Using Cecectomized Rooster Assay		
Predicted MP allowable milk, kg	46.0	50.3

Discussion

One purpose of this study was to compare the Ross assay ID predictions with the values determined by another commonly accepted ID assay, the cecectomized rooster bioassay. The Ross IVNIDA previously provided ID that allowed for a more to accurate prediction of MP allowable milk yield in previous studies in dairy cattle (Higgs et al. 2013; Gutierrez-Botero 2015), but testing it against the ID predicted by other assays, such as the cecetomized rooster assay, would allow our lab to fine tune the methodology of the Ross assay if needed. Feather meal was used for this study because the cecectomized rooster assay indicated that it has a higher ID than what was determined by the Ross IVNIDA. Two diets were formulated to have the same ME allowable milk and total N content of the diet, but different ID based on the Ross assay, allowing us to test for differences in production measures in dairy cattle fed these two different diets.

The results of this study support previous observations that the values from the Ross assay when used in the CNCPS model provided reasonable estimates of MP allowable milk under N limiting conditions (Gutierrez-Botero 2015). The differences in milk yield, ECM, protein, and fat suggest that the cattle fed the HID diet were being supplied more MP, and were less limited by protein in their diet than the cattle fed the LID diet. This is in agreement with the measurements of uN in FM and BM using the predictions of the Ross assay, and the resulting predictions of ID of these feeds. Despite the unexpected drop in feed offered to the cattle during the experiment resulting lower DMI for both treatments, the CNCPS was able to predict MP allowable milk yield using the values generated from the Ross assay and other usual inputs such as feed chemistry, cattle characteristics (Table 5).

A problem occurred during the trial where for a three week period beginning around January 1, the diet was incorrectly mixed and fed due to a software error in the Feed Watch software which

automatically assigns the amounts of each ingredient to be placed in the feed mixer. This altered the intake of the cattle because the protein mixes were not provided at the level originally formulated for both treatment diets but because the error was identical for both treatments, comparisons could still be made as diets were isocaloric and isonitrogenous. Whether the reason for the malfunction was incorrect entry of inputs or error in the system is unknown. The subsequent variability in the data is likely the reason that differences in milk yield between cattle fed HID vs LID diets was identified as a trend and not significant. The data showed that the milk yield remained different between treatments throughout the trial but narrowed in the later weeks. The initial change in milk yield was approximately 2 kg/d and that difference was not large enough to reach significance given the shift in milk yield during the period cattle were fed incorrectly mixed feed. While MP allowable milk was overestimated for the HID cattle, this prediction was within reason and was most likely due to the inability of the CNCPS model to predict the decrease in DMI, leading to over-prediction based on the previous level of intake. Actual ME and MP allowable milk yield for the inputs from the Ross assay (Table 5), and deviated substantially from what was predicted by CNCPS using the cecectomized rooster assay. This deviation was especially pronounced in the LID diet. These findings demonstrate that the cecectomized rooster assay is not valid for predicting the ID of feeds fed to ruminants. The assay might be able to differentiate digestibilities among feeds, but the absolute differences are not applicable to cattle as was implied in the paper published by Boucher et al. (2009). Due to the presence of the enzyme elastase in the rooster digestive tract, there is an expected increase in the digestibility of keratinized proteins within the rooster assay which is not found within the ruminant. This would increase the digestibility of animal proteins such as FM beyond what is feasible in the lactating dairy cow.

While FM is less digestible by cattle than BM, it is also a less expensive protein to add to diets. To compare, FM is $537/metric ton when imported to the farm as opposed to BM, which is $913/ton. Their CP values are marginally different because FM has a lower total N content. Blood meal is 98.3% CP and FM is 85% CP. Using the results from the Ross assay, uN is 25.4% N for BM and 45.4% N for FM. For every ton of BM, there is 72.9% available protein, making total protein available per ton 709 kg. Feather meal contains about 54.6% available protein, and therefore the total protein available per ton is 421 kg. When the cost is adjusted per unit of available protein the two proteins are remarkably similar at $1.17/kg for BM and $1.16/kg for FM. Thus the FM is not an inexpensive feed when assessed based on N availability and digestibility and the BM looks much more attractive, especially given the amount of FM you would have to add to a diet to equal the available protein in BM.

In addition to the financial aspects of different protein sources for diets, BM might be a preferable alternative to feeding FM from an environmental standpoint. Because the BM has greater ID than FM, less is needed in the diet resulting in less indigestible N excreted and a potential reduction in the environmental impact of milk production. As emphasis on N in the environment continues to increase, farmers might be able to consider the digestibility of N sources in feeds to decrease the environmental impact of dairy production.

Conclusions

The results of this study support the hypothesis that the Ross assay can accurately predict uN and ID in feeds consumed by lactating dairy cattle. Despite the decrease in DMI observed throughout the feeding trial and the subsequent decrease in milk yield, the cattle fed the HID diet formulated with BM maintained a higher yield, ECM, and components in the milk than their LID counterparts. Given that the cecetomized rooster assay would have predicted the opposite effect, these results suggest that the cecetomized rooster assay may not be the most effective way to measure N digestibility of feeds for the lactating dairy cow. This is possibly due to the different enzymes present in the intestines of the rooster. The cow is potentially unable to break down the keratin in the FM because it lacks the enzymes found within the chicken digestive tract. Therefore, as a source of animal protein, FM is not the most digestible feed to include in the diets of lactating dairy cattle. Additionally, the actual economic value of using FM in diets of lactating cattle is less than that of using BM, because the lower cost does not make up for the lower digestibility.

Literature Cited

AOAC International. (1999). Official method 971.09. Official Methods of Analysis of AOAC International, 16th edition 5th revision. AOAC International: Gaithersburg, MD 20877-2417, USA.

AOAC International. (2006). Official methods of analysis of AOAC International. AOAC International, Gaithersburg, MD MD 20877-2417, USA.

Boucher, S. E., S. Calsamiglia, C. M. Parsons, H. H. Stein, M. D. Stern, P. S. Erickson, P. L. Utterback, and C. G. Schwab. (2009). Intestinal digestibility of amino acids in rumen undegradable protein estimated using a precision-fed cecectomized rooster bioassay: I. Soybean meal and soyplus. J. Dairy Sci., 92, 4489-4498.

Calsamiglia, S., and Stern, M. D. (1995). A three-step in vitro procedure for estimating intestinal digestion of protein in ruminants. J. Anim. Sci., 73, 1459–1465.

DuBois, M. K. A. Gilles, J. K. Hamilton, P. A. Rebers, and Smith F. (1956). Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Ferraro, V., Anton, M., and Santé-Lhoutellier, V. (2016). The "sisters" α-helices of collagen, elastin and keratin recovered from animal by-products: Functionality, bioactivity and trends of application. Trends in Food Science and Technology, 51, 65–75.

Fessenden, S. W., Hackmann, T. J., Ross, D. A., Foskolos, A., and Van Amburgh, M. E. (2017). Ruminal bacteria and protozoa composition, digestibility, and amino acid profile determined by multiple hydrolysis times. J. Dairy Sci., 100, 7211–7226.

Fox, D.G., Tylutki, T.P., Tedeschi, L.O., Van Amburgh, M. E., Chase, L. E., Pell, A. P., Overton, T. R., and Russell, J. B. (2004). The Net Carbohydrate and Protein System

model for evaluating herd nutrition and nutrient excretion. Anim. Sci. Feed Tech. 112, 29-78.

Goering, H. K., C. H. Gordon, R. W. Hemken, D. R. Waldo, P. J. Van Soest, and L. W. Smith. (1972). Analytical estimates of nitrogen digestibility in heat damaged forages. J. Dairy Sci., 55, 1275-1280.

Gutierrez-Botero, M. (2015). Determination of feed unavailable nitrogen to increase productive efficiency in high producing dairy cattle. M.S Thesis. Dept. of Animal Sci. Cornell University, Ithaca, NY.

Hall, M. B. (2015). Determination of dietary starch in animal feeds and pet food by an enzymatic-colorimetric method: Collaborative study. J. AOAC Int., 98, 397-409.

Higgs, R. J., L. E. Chase, and M. E. Van Amburgh. (2012). Development and evaluation of equations in the Cornell Net Carbohydrate and Protein System to predict nitrogen excretion in lactating dairy cows. J. Dairy Sci.., 95, 2004–2014.

Higgs, R. J., Chase, L. E., Ross, D. A., and Van Amburgh, M. E. (2015). Updating the Cornell Net Carbohydrate and Protein System feed library and analyzing model sensitivity to feed inputs. J. Dairy Sci., 98, 6340–6360.

Hristov, A. N., and Ropp, J. K. (2003). Effect of dietary carbohydrate composition and availability on utilization of ruminal ammonia nitrogen for milk protein synthesis in dairy cattle. J. Dairy Sci., 86, 2416–2427.

Hvelplund, T., and J. Madsen. (1995). Protein utilization in ruminants. Protein metabolism and nutrition. Pp. 83-93. In: Proc. 7th Int. Symp. EAAP Publ. 81. Estacao Zootecnica Nacional, Santarem, Portugal.

Kauffman, A. J., and St-Pierre, N. R. (2001). The relationship of milk urea nitrogen to urine nitrogen excretion in Holstein and Jersey cattle. J. Dairy Sci., 84, 2284–2294.

National Research Council. (2001). Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy of Science. Washington, DC.

Ouellet, D. R., Berthiaume, R., Holtrop, G., Lobley, G. E., Martineau, R., and Lapierre, H. (2010). Effect of method of conservation of timothy on endogenous nitrogen flows in lactating dairy cattle. J. Dairy Sci., 93, 4252–4261.

Parsons, C. M. (1985). Influence of caecectomy on digestibility of amino acids by roosters fed distillers' dried grains with solubles. J. Agric. Sci., 104, 469.

Pichard, D.C. and Van Soest, P.J. (1977). Protein solubility of ruminant feeds. In: Proc. Cornell Nutr. Conf. Feed Manuf., Pp. 91-98. Syracuse. NY.

Reynolds, C. K., and Kristensen, N. B. (2008). Nitrogen recycling through the gut and the nitrogen economy of ruminants: an asynchronous symbiosis. J. Anim. Sci., 86, E293-305.

Ross, D. A., Gutierrez-Botero, M., and Van Amburgh, M. E. (2013). Development of an in vitro intestinal digestibility assay for ruminant feeds. In: Proc. Cornell Nutr. Conf. Feed Manuf., Pp. 191-202. Syracuse, NY.

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., and Russell, J. B. (1992). A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci., 70, 3562–3577.

Titgemeyer, E., Merchen, N., Han, Y., Parsons, C., and Baker, D. (1990). Assessment of intestinal amino acid availability in cattle by use of the precision-fed cecectomized rooster assay. J. Dairy Sci., 73, 690–693.

Tyrrell, H.F. and Reid, J.T. (1965). Prediction of the energy value of cow's milk. J. Dairy Sci., 48, 1215-23.

Van Amburgh, M. E., Collao-Saenz, E. A., Higgs, R. J., Ross, D. A., Recktenwald, E. B., Raffrenato, E., … Foskolos, A. (2015). The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. J. Dairy Sci., 98, 6361–6380.

Van Amburgh, M. E., Ortega, A. F., Fessenden, S. W., Ross, D. A., and Lapierre, P. A. (2017). The amino acid content of rumen microbes, feed, milk and tissue after multiple hydrolysis times and implications for the CNCPS. In: Proc. Cornell Nutr. Conf. Feed Manuf. Pp. 125-140. Syracuse, NY.

Van Soest P. J., J. B. Robertson, B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74, 3583-97.

Van Vuuren, A.M and J.A.C. Meijs. (1987). Effects of herbage composition and supplement feeding on the excretion of nitrogen in dung and urine by grazing dairy cows. in: animal manure on grassland and fodder crops. fertilizer or waste? Pp. 17-25, Martinus Nijhoff Publishers, Dordrecht, Netherlands,.